Validation of the 12-Gene Colon Cancer Recurrence Score in NSABP C-07 As a Predictor of Recurrence in Patients With Stage II and III Colon Cancer Treated With Fluorouracil and Leucovorin (FU/LV) and FU/LV Plus Oxaliplatin

Greg Yothers, Michael J. O'Connell, Mark Lee, Margarita Lopatin, Kim M. Clark-Langone, Carl Millward, Soonmyung Paik, Saima Sharif, Steven Shak, and Norman Wolmark

Greg Yothers, Michael J. O'Connell, Soonmyung Paik, Saima Sharif, and Norman Wolmark, National Surgical Adjuvant Breast and Bowel Project (NSABP) Operations and Biostatistical Centers; Greg Yothers, University of Pittsburgh Graduate School of Public Health; Saima Sharif and Norman Wolmark, Allegheny Cancer Center at Allegheny General Hospital, Pittsburgh, PA; Mark Lee, Margarita Lopatin, Kim M. Clark-Langone, and Steven Shak, Genomic Health, Redwood City, CA.

Published online ahead of print at www.jco.org on November 12, 2013.

Supported by Public Health Service Grants No. U10-CA-12027, U10-CA-69651, U10-CA-37377, and U10-CA-69974 from the National Cancer Institute, Department of Health and Human Services, and Sanofi-Synthelabo.

Related work presented in part at the 48th Annual Meeting of the American Society of Clinical Oncology, June 1-5, 2012, Chicago, IL.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical trial information: NCT00004931.

Corresponding author: Greg Yothers, PhD, NSABP Biostatistical Center and the University of Pittsburgh Graduate School of Public Health, Department of Biostatistics, 201 North Craig St, Ste 350, Pittsburgh, PA 15213; e-mail: yothers@nsabp.pitt.edu.

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0732-183X/13/3136w-4512w/\$20.00

DOI: 10.1200/JCO.2012.47.3116

ABSTRACT

Purpose

Accurate assessments of recurrence risk and absolute treatment benefit are needed to inform colon cancer adjuvant therapy. The 12-gene Recurrence Score assay has been validated in patients with stage II colon cancer from the Cancer and Leukemia Group B 9581 and Quick and Simple and Reliable (QUASAR) trials. We conducted an independent, prospectively designed clinical validation study of Recurrence Score, with prespecified end points and analysis plan, in archival specimens from patients with stage II and III colon cancer randomly assigned to fluorouracil (FU) or FU plus oxaliplatin in National Surgical Adjuvant Breast and Bowel Project C-07.

Methods

Recurrence Score was assessed in 892 fixed, paraffin-embedded tumor specimens (randomly selected 50% of patients with tissue). Data were analyzed by Cox regression adjusting for stage and treatment.

Results

Continuous Recurrence Score predicted recurrence (hazard ratio for a 25-unit increase in score, 1.96; 95% CI, 1.50 to 2.55; $P < .001$), as well as disease-free and overall survival (both $P < .001$). Recurrence Score predicted recurrence risk ($P = .001$) after adjustment for stage, mismatch repair, nodes examined, grade, and treatment. Recurrence Score did not have significant interaction with stage ($P = .90$) or age ($P = .76$). Relative benefit of oxaliplatin was similar across the range of Recurrence Score (interaction $P = .48$); accordingly, absolute benefit of oxaliplatin increased with higher scores, most notably in patients with stage II and IIIA/B disease.

Conclusion

The 12-gene Recurrence Score predicts recurrence risk in stage II and stage III colon cancer and provides additional information beyond conventional clinical and pathologic factors. Incorporating Recurrence Score into the clinical context may better inform adjuvant therapy decisions in stage III as well as stage II colon cancer.

J Clin Oncol 31:4512-4519. © 2013 by American Society of Clinical Oncology

INTRODUCTION

Adjuvant therapy decisions require the physician and patient to balance an understanding of the patient's likelihood of recurrence and expected absolute treatment benefit with the patient's comorbidities, life expectancy, and toxicities of therapy. For resected colon cancer patients, adding oxaliplatin to adjuvant fluoropyrimidine chemotherapy reduces risk of recurrence and mortality, as evidenced by the National Surgical Adjuvant Breast and Bowel Project (NSABP) protocol C-07,^{1,2} Multicenter In-

ternational Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC),^{3,4} and Xeloda and Oxaliplatin for Adjuvant Therapy (XELOXA)⁵ randomized clinical trials. The addition of oxaliplatin to adjuvant fluoropyrimidine chemotherapy results in an approximately 20% relative risk reduction for disease-free survival (DFS), translating to a 6% absolute benefit at 5 years, with attenuated absolute benefit for overall survival (OS). Moreover, inclusion of oxaliplatin considerably augments the toxicity of adjuvant therapy, including the

prospect of long-term peripheral neuropathy. Concerns over oxaliplatin-induced neuropathy have prompted multiple clinical trials evaluating interventions to mitigate this toxicity.^{6,7}

With a need to balance limited absolute benefit against significant toxicity, the prevailing paradigm has focused on directing adjuvant therapy to patients at higher recurrence risk, with the expectation of larger absolute treatment benefit.^{8,9} Although stage does not predict for the relative benefit of fluorouracil (FU)/leucovorin (LV) or oxaliplatin, stage III patients, compared with stage II patients, have higher recurrence risk and larger absolute treatment benefit.^{2,10} These differences in the magnitude of risk and absolute benefit have led to the recommendation of adjuvant oxaliplatin-containing therapy for stage III patients and a controversy regarding adjuvant therapy for stage II patients.^{11,12} Within stage II, oxaliplatin is considered for high-risk features including T4 stage, high tumor grade, lymphovascular invasion, or inadequate nodal sampling, based on larger absolute benefit observed in an exploratory analysis from MOSAIC.^{4,12}

However, these conventional clinical and pathologic markers lack standardization and prospective validation and do not provide adequate individualized assessment of recurrence risk after FU/LV treatment or quantification of absolute benefit from the addition of oxaliplatin. At present, markers reported to have association with oxaliplatin benefit (eg, *ERCC1*) remain exploratory, lacking prospective validation in specimens from randomized adjuvant therapy trials.¹³⁻¹⁶ As such, standardized, quantitative tools that improve assessment of recurrence risk and absolute treatment benefit are needed to prevent both over- and undertreatment of patients.

To address this need, the 12-gene *Oncotype DX* Colon Cancer Recurrence Score Assay (Genomic Health, Redwood City, CA) was developed using tumor gene expression data from 1,851 patients with stage II and III colon cancer in four large, independent development studies as a predictor of recurrence risk.¹⁷ In the prospectively designed Quick and Simple and Reliable (QUASAR) validation study, with tumor samples from 1,436 patients with stage II colon cancer randomly assigned to surgery alone or surgery followed by adjuvant FU/LV, the continuous Recurrence Score was significantly associated with recurrence risk after surgery¹⁸ and predicted recurrence risk beyond mismatch repair (MMR), T stage, tumor grade, number of nodes examined, and lymphovascular invasion. The value of Recurrence Score was most evident in patients with T3N0 MMR-proficient tumors, where conventional risk factors demonstrated limited utility. Moreover, because relative benefit of adjuvant FU/LV was found to be independent of Recurrence Score in QUASAR, patients with a higher Recurrence Score are expected to derive larger absolute benefits with adjuvant treatment.¹⁸ The relationship of Recurrence Score to recurrence risk was confirmed in an independent, prospectively defined study of 690 patients with stage II colon cancer from Cancer and Leukemia Group B (CALGB) 9581.¹⁹

The Recurrence Score is a standardized, quantitative, reverse transcriptase polymerase chain reaction (RT-PCR) assay that measures the expression of 12 genes (seven recurrence and five reference genes) in fixed, paraffin-embedded (FPE) primary colon tumor tissue.^{17,18,20} The recurrence genes integrate the activity of two key biologic pathways, cell cycle control and stromal response. Although differences between stage II and stage III colon cancer were observed for a small subset of genes, expression of the vast majority of 375 cancer-related genes, including the Recurrence Score genes, was remarkably similar across stage. As expected, Recurrence Score showed

no evidence of interaction with stage in the development studies¹⁷ and would be expected to perform similarly in stage II and III colon cancer.

Prospective collection of FPE tumor tissues from NSABP C-07 provides an ideal opportunity to study the pathologic and molecular determinants of outcome in a large randomized trial that evaluated adjuvant oxaliplatin for patients with stage II and III colon cancer. We conducted an independent, prospectively designed clinical validation study of Recurrence Score, with prespecified end points, methods, and analysis plan, in archival specimens from patients with stage II and III colon cancer randomly assigned to FU or FU plus oxaliplatin in NSABP C-07.

METHODS

Patients and Tissue

Patients with stage II and III colon cancer randomly assigned and treated with FU/LV or FU/LV + oxaliplatin in NSABP C-07 with available tumor tissue and clinical follow-up were divided into two equally sized cohorts using random sampling stratified on recurrence status and nodal status (zero, one to three, and $\geq$ four positive lymph nodes, corresponding to stage II, IIIA/B, and IIIC, respectively [American Joint Committee on Cancer staging, sixth edition]).²¹ The first cohort served as the validation study for Recurrence Score described here. Availability of two cohorts from C-07 enabled a parallel effort to develop and validate a novel multigene oxaliplatin benefit predictor to be reported separately.

Pathology

Pathologic T stage, number of nodes examined, and number of nodes involved by carcinoma were obtained from the NSABP clinical database. MMR status was assessed by immunohistochemistry for hMLH1 and hMSH2. Tumor grade was centrally assessed²² by an academic surgical pathologist specialized in GI pathology.

Gene Expression

RNA was extracted from five 5- μ m sections from a single representative archived FPE tumor block for each patient, quantified, and analyzed by RT-PCR, using a standardized, analytically validated process.²⁰ Recurrence Score values were calculated using the prespecified genes and algorithm. Prespecified Recurrence Score groups were as follows: low (score, < 30), intermediate (score, 30 to 40), and high (score, ≥ 41).¹⁸ All centrally performed pathology and laboratory procedures were prespecified and conducted without knowledge of patient clinical characteristics or outcomes.

Statistical Methods

The primary end point was recurrence-free interval (RFI), defined as time from random assignment to first colon cancer recurrence. Evidence of recurrence at death (eg, on autopsy) was considered a recurrence event. Patients who died without evidence of recurrence were censored at the time of death. Patients alive without recurrence were censored at last follow-up. Second primary cancers were neither censored nor treated as events. Secondary end points included DFS, which was defined as time from random assignment to first colon cancer recurrence, any second primary cancer, or death from any cause, and OS, which was defined as time from random assignment to death from any cause.

In the primary analysis, Cox proportional hazards regression with adjustment for stage (II, IIIA/B, or IIIC) and randomly assigned treatment was used to evaluate the association between continuous Recurrence Score and outcomes with the likelihood ratio test. Contribution of Recurrence Score to prediction of recurrence risk beyond prespecified clinically important pathologic covariates was assessed. Interactions of Recurrence Score with each covariate were assessed by sequentially adding interaction terms to the model. A two-sided $P < .025$ was considered significant for the primary analysis, and $P < .05$ was considered significant for other analyses. Assessment of proportional hazards was performed by examining the relationship between scaled Schoenfeld residuals and time.^{23,24} Kaplan-Meier and cumulative incidence

Fig 1. Flowchart showing patient and sample accounting in National Surgical Adjuvant Breast and Bowel Project (NSABP) C-07; 4.5% of patients eligible for the study did not have death certificates.

analyses were used to characterize the relationship between RFI and Recurrence Score groups. Analyses used SAS version 9.2 (SAS Institute, Cary, NC) or R version 2.14.1 (cmpsrk package; www.r-project.org).

The study protocol, with prespecified end points, laboratory methods, and analysis plan, was finalized on May 31, 2011. RT-PCR analysis of tumor specimens commenced on July 8, 2011, and laboratory and clinical data were merged on November 11, 2011.

RESULTS

Patient Characteristics

Tumor tissue was available for 1,860 (77%) of 2,409 eligible stage II/III patients from the NSABP C-07 trial, from which 929 patient specimens (50%) were randomly selected for this study. On the basis of prespecified criteria for pathology and laboratory processing, 37 specimens (4.0%) were excluded, mainly because of ineligible histology (Fig 1). The final evaluable data set contained 892 patients, with recurrences in 31 (12%) of 264 stage II patients and 214 (34%) of 628 stage III patients.

Treatment arms were well-balanced with respect to clinical and pathologic covariates (Table 1). Patients were mostly white and male, with an average age of 58 years (including 17% over age 70 years) and a median of 13 nodes examined (approximately 60% with ≥ 12 nodes). Consistent with previous reports,²⁵ MMR deficiency was more frequent in stage II than in stage III (Table 1). Demographic and clinical characteristics of this study cohort closely mirrored the overall C-07 trial and were similar to the patient population not included in this study (Appendix Table A1, online only). As with the overall C-07 trial,² absolute benefit from addition of oxaliplatin to FU was most apparent for stage III patients, although the interaction of treatment and stage was not significant ($P = .36$; Appendix Fig A1, online only), suggesting that the relative benefit of oxaliplatin is similar by stage.

Table 1. Demographic and Baseline Clinical Characteristics of Patients in NSABP C-07

Characteristic	FU (n = 449)		FU + Oxaliplatin (n = 443)		Total (N = 892)	
	No. of Patients	%	No. of Patients	%	No. of Patients	%
Age, years						
< 60	214	47.7	230	51.9	444	49.8
60-69	164	36.5	134	30.2	298	33.4
≥ 70	71	15.8	79	17.8	150	16.8
Sex						
Male	258	57.5	255	57.6	513	57.5
Female	191	42.5	188	42.4	379	42.5
Race						
White	400	89.1	383	86.7	783	87.9
Black	22	4.9	26	5.9	48	5.4
Other	27	6.0	33	7.5	60	6.7
Year of surgery						
2000	153	34.1	155	35.1	308	34.6
2001	143	31.8	142	32.1	285	32.0
2002	153	34.1	145	32.8	298	33.4
Nodal stage						
Stage II	131	29.2	133	30.0	264	29.6
Stage IIIA/B	208	46.3	201	45.4	409	45.9
Stage IIIC	110	24.5	109	24.6	219	24.6
T stage						
T4 in stage II	7	5.3	10	7.5	17	6.4
T3-4 in stage III	271	85.2	261	84.2	532	84.7
MMR						
MMR-D in stage II	22	21.0	17	15.7	39	18.3
MMR-D in stage III	19	7.8	26	10.3	45	9.1
No. of nodes examined						
≥ 12 in stage II	84	64.1	80	60.2	164	62.1
≥ 12 in stage III	188	59.1	196	63.2	384	61.1
Tumor grade, high	121	26.9	104	23.5	225	25.2

Abbreviations: FU, fluorouracil; MMR, mismatch repair; MMR-D, mismatch repair deficiency; NSABP, National Surgical Adjuvant Breast and Bowel Project.

Association of Recurrence Score With RFI, DFS, and OS

The continuous Recurrence Score (median score, 33; interquartile range, 25 to 41) was significantly associated with RFI (hazard ratio [HR] for a 25-unit increase in score, 1.96; 95% CI, 1.50 to 2.55; $P < .001$; equivalent to HR of 1.55 per interquartile range [16-unit increase]) after adjusting for stage and treatment. Recurrence Score performed similarly in stage II, IIIA/B, and IIIC patients (interaction $P = .90$). Wide ranges of recurrence risks quantified by Recurrence Score were observed for each stage based on the primary analysis model (Fig 2), with substantial overlap in risk between stages across the range of Recurrence Score values.

The predefined high Recurrence Score group (26% of patients) had higher recurrence risk than the low score group (39% of patients; HR, 2.11; 95% CI, 1.54 to 2.88; $P < .001$) after adjustment for stage and treatment. On the basis of the primary Cox model, average 5-year recurrence risk rates in FU-treated patients by Recurrence Score group (low, intermediate, and high) were as follows: stage II, 9% (95% CI, 6% to 13%), 13% (95% CI, 8% to 17%), and 18% (95% CI, 12% to

Fig 2. Relationship between the continuous Recurrence Score (RS) and 5-year recurrence risk by stage and treatment in National Surgical Adjuvant Breast and Bowel Project C-07. Thick lines represent fluorouracil (FU)-treated patients, thin lines represent FU + oxaliplatin-treated patients. Blue, gold, and gray colors represent stage II, IIIA/B, and IIIC, respectively. A rug plot depicting the distribution of RS is included at the bottom of the figure, and an estimated normal distribution of scores is provided below. The proportional hazards assumption held ($P = .20$ for the test of nonzero slope of Schoenfeld residuals v time). The relationship between continuous RS and the log hazard of recurrence was linear ($P = .84$ for the test of nonlinearity).

25%); stage IIIA/B, 21% (95% CI, 16% to 26%), 29% (95% CI, 24% to 34%), and 38% (95% CI, 30% to 46%); and stage IIIC, 40% (95% CI, 32% to 48%), 51% (95% CI, 43% to 59%), and 64% (95% CI, 55% to 74%), respectively. No significant interaction between Recurrence Score and treatment was identified ($P = .48$), and the model estimate suggests that relative treatment benefit is nondecreasing with increasing score, indicating that higher absolute oxaliplatin benefit is obtained at higher Recurrence Score values. In agreement with these findings from the Cox model, across stage, Kaplan-Meier analysis demonstrated higher absolute benefit of oxaliplatin in the high Recurrence Score group, overall and within each stage, compared with the low Recurrence Score group (Fig 3; Appendix Table 2, online only). Similar findings were obtained using post hoc analyses accounting for competing risks (Appendix Fig A2, online only).

An ad hoc analysis of the primary model restricted to stage III patients confirms that the continuous Recurrence Score is associated with RFI in stage III patients, with an HR of 1.90 (95% CI, 1.43 to 2.53) for a 25-unit increase in the score ($P < .001$). The small number of events in stage II patients in this cohort precludes a suitably powered subset analysis.

Recurrence Score was significantly associated with DFS (HR for a 25-unit increase in score, 1.60; 95% CI, 1.28 to 1.99; $P < .001$) and OS

(HR, 1.89; 95% CI, 1.46 to 2.44; $P < .001$), consistent with the observation that most deaths (77%) observed in this study were preceded by recurrence and thus colon cancer specific.

Contribution of Recurrence Score Beyond Conventional Prognostic Factors

Pathologic T stage, MMR status, number of nodes examined, and tumor grade were significantly associated with RFI after adjusting for stage and treatment when analyzed individually (Appendix Table A3, online only). Neither age nor sex was significantly associated with recurrence.

The continuous Recurrence Score predicted recurrence risk ($P = .001$) after adjustment for N stage, T stage, MMR status, nodes examined, grade, and treatment (Table 2). Consistent with previous studies,^{18,19} N and T stage, MMR, and Recurrence Score were the most significant covariates. No interaction of Recurrence Score with any of the covariates was observed (all $P > .25$). Accordingly, Recurrence Score performance in standard-risk, MMR-proficient patients was similar to the overall primary analysis result (HR for a 25-unit increase in score, 1.99; 95% CI, 1.45 to 2.73). A wide range of Recurrence Score values was observed at each covariate level (Fig 4), illustrating that the Recurrence Score cannot be predicted by conventional measures. Age, when assessed as a continuous variable, was not significantly associated with recurrence risk, but its interaction with treatment was borderline significant ($P = .056$), suggesting lower benefit of oxaliplatin with older age, consistent with the parent trial.² Interaction of age and Recurrence Score in this expanded model was not significant ($P = .81$). Association of Recurrence Score with risk of recurrence was not affected by adjustment for age and interaction of age and treatment (HR for a 25-unit increase in score, 1.93; 95% CI, 1.49 to 2.52).

DISCUSSION

In this prospectively designed study, the 12-gene Recurrence Score was validated in patients with stage II and III colon cancer from NSABP C-07 treated with FU/LV $\pm$ oxaliplatin as a predictor of recurrence risk, providing information beyond conventional clinical and pathologic factors. NSABP C-07 provided an opportunity to study Recurrence Score and its association with recurrence risk in the context of adjuvant treatment with FU and FU + oxaliplatin. Consistency of these results with previous large validation studies in QUASAR and CALGB 9581 reaffirms the connection of Recurrence Score with the metastatic potential of colon cancer.^{18,19}

A better understanding of recurrence risk should have clinical relevance for patients and physicians considering oxaliplatin therapy for stage II and III colon cancer. Adding oxaliplatin to fluoropyrimidine chemotherapy results in increased grade 3 or 4 cytopenias and diarrhea, as well as both acute and chronic neurotoxicity.^{2,4,6,7} In both NSABP C-07 and MOSAIC, peripheral neuropathy of any grade was observed in more than 60% of patients during oxaliplatin treatment, with 22% of patients reporting symptoms 18 months from random assignment in NSABP C-07 and 15% of patients reporting symptoms at 4 years in MOSAIC.^{2,4} Recent studies focused on assessing the long-term consequences of oxaliplatin treatment suggest that chronic neurotoxicity is likely more prevalent than previously reported.^{26,27} The risk of neuropathy, which can significantly impact long-term patient quality of life, must be balanced with the likelihood of recurrence and absolute treatment benefit with oxaliplatin (approximately

Fig 3. Recurrence-free interval for (A) all patients and (B) stage II, (C) stage IIIA/B, and (D) stage IIIC patients by Recurrence Score (RS) groups and treatment in National Surgical Adjuvant Breast and Bowel Project C-07. Thick lines represent fluorouracil (FU) –treated patients, and thin lines represent FU + oxaliplatin (FU + Ox) –treated patients. Gray, gold, and blue colors represent low, intermediate (int), and high RS groups, respectively. Across all patients (A), FU-treated patients in the high RS group had higher risk of recurrence compared with patients treated with FU + Ox (log-rank $P = .016$), whereas patients in the low RS group had similar recurrence risks (log-rank $P = .98$). Numbers of deaths without recurrence were 20, 30, and 12 in stage II, IIIA/B, and IIIC, respectively.

6% for DFS in unselected stage III patients).⁹ It is important to note that the Recurrence Score is not a predictor of oxaliplatin treatment efficacy and therefore cannot identify patients for whom oxaliplatin has no benefit. The Recurrence Score, in combination with stage,

provides better discrimination of recurrence risk and thus may enable better discrimination of absolute benefit as a function of risk, with patients with a high Recurrence Score expected to derive larger absolute treatment benefit than patients with low Recurrence Score. Higher recurrence risks at higher Recurrence Score along with potentially higher absolute treatment benefit may be helpful, in selected patients, for the important discussions that patients and physicians have regarding estimates of likely benefit and risk with different treatment options. In this regard, a survey of patients with colon cancer revealed that knowledge of recurrence risk and absolute benefit can strongly influence adjuvant treatment decisions, with more patients opting to receive therapy over a range of increasing levels of absolute benefit (tested values of absolute benefit: 1%, 3%, 5%, and 10%).²⁸

The Recurrence Score may have particular impact for older patients, given evidence for reduced oxaliplatin benefit in those older than age 70 years in NSABP C-07 and in the Adjuvant Colon Cancer Endpoints (ACCENT) pooled analysis of adjuvant trials.^{2,27} In this study, a borderline interaction of age and treatment was observed ($P = .056$), consistent with results from the overall C-07 study. However, the interaction of Recurrence Score and age was not significant ($P = .81$), indicating that the performance and biologic relevance of Recurrence Score was similar across age groups. Recurrence Score

Table 2. Multivariable Analysis: Relationship of Clinical and Pathologic Covariates With Risk of Recurrence in NSABP C-07

Variable	HR	95% CI	P
Stage (by nodal status)			< .001
II v IIIC	0.97	0.55 to 1.71	
IIIA/B v IIIC	2.07	1.16 to 3.68	
Treatment: FU + Ox v FU	0.82	0.64 to 1.06	.12
MMR: MMR-D v MMR-P	0.27	0.12 to 0.62	< .001
T stage: T4 stage II and T3-4 stage III v T3 stage II and T1-2 stage III	3.04	1.84 to 5.02	< .001
Nodes examined: < 12 v ≥ 12	1.51	1.17 to 1.95	.002
Tumor grade: high v low	1.36	1.02 to 1.82	.041
Recurrence Score: continuous per 25 units	1.57	1.19 to 2.08	.001

Abbreviations: FU, fluorouracil; HR, hazard ratio; MMR, mismatch repair; MMR-D, mismatch repair deficiency; MMR-P, mismatch repair proficiency; NSABP, National Surgical Adjuvant Breast and Bowel Project; Ox, oxaliplatin.

Fig 4. Distribution of the Recurrence Score (RS) by levels of covariates in National Surgical Adjuvant Breast and Bowel Project C-07: (A) stage, (B) nodes examined, (C) age, (D) mismatch repair (MMR) status, (E) T stage, and (F) tumor grade. *P* values are from an analysis of variance test for association of the continuous RS with the levels of the covariate. Gray horizontal lines represent cut points for low and high RS groups (RS = 30 and 41, respectively); percentage of patients in each RS group at each level of the covariate is shown.

performed consistently across stage II, IIIA/B, and IIIC patients (interaction $P = .90$), confirming observations in development studies.^{17,28} Wide distributions of Recurrence Score in patient subgroups defined by conventional covariates, such as age, N stage, T stage, MMR status, tumor grade, and nodes examined, as well as the lack of interaction between Recurrence Score and these factors, reaffirm that the Recurrence Score provides independent information beyond conventional factors.

In multivariable analyses, stage (T and N stage) was confirmed as a powerful discriminator of recurrence risk; however, it is clear that Recurrence Score can explain much of the heterogeneity within each stage. MMR status was also strongly associated with recurrence, with MMR deficiency predicting lower recurrence risk.^{25,29} The low frequency of MMR-deficient tumors in stage III and overall in this study, together with the low event rate in this population, precludes refined estimates of recurrence risk for these patients; future studies, including

pooled analyses across multiple trials, should address this issue. Although number of nodes examined was also associated with recurrence, inadequate nodal sampling is increasingly uncommon in clinical practice. Finally, other pathologic factors, such as tumor grade, remain limited by lack of standardization and validation in prospectively designed studies, as well as inconsistent results in the literature.^{8,30} Taken together, the Recurrence Score is likely to have its greatest clinical value when incorporated into the clinical context for each patient, particularly in conjunction with stage and potentially MMR status.

Within stage III, Recurrence Score should be most informative for the subset of stage IIIA/B patients for whom higher absolute treatment benefit may be required to justify oxaliplatin usage. For stage II, estimates of absolute treatment benefit increased with higher Recurrence Score values in both the Cox model and Kaplan-Meier analyses; given the small number of events in stage II, however, the Cox model estimates, leveraging all of the study data, represent a more appropriate interpretation of the data. Further refinement of the ability to discriminate oxaliplatin benefit could impact treatment decisions for a broader range of patients. In parallel with the validation of Recurrence Score described here, exploratory analyses are being conducted in this first cohort of C-07 to identify new genes, beyond Recurrence Score, that are associated with sensitivity and resistance to oxaliplatin (to be reported separately).

Moving forward, the Recurrence Score may enable risk-stratified clinical trial designs in more homogeneously defined low- and high-risk patient populations in the adjuvant setting. Patients with high recurrence risk after FU/LV + oxaliplatin could be studied as candidates for more aggressive therapy, including the addition of targeted agents. In the near term, incorporation of Recurrence Score into the clinical context should enable patients with stage II and III colon cancer to make more informed treatment decisions, balancing potential toxicities of therapy with more refined knowledge of recurrence risk and absolute treatment benefit. Certain stage II and stage IIIA/B patients with low Recurrence Score disease may elect to forego oxalip-

latin treatment as a result of their low risk of recurrence and small absolute benefit with oxaliplatin.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Mark Lee, Genomic Health (C); Margarita Lopatin, Genomic Health (C); Kim M. Clark-Langone, Genomic Health (C); Steven Shak, Genomic Health (C) **Consultant or Advisory Role:** Norman Wolmark, Genomic Health (U), sanofi-aventis (U) **Stock Ownership:** Mark Lee, Genomic Health; Margarita Lopatin, Genomic Health; Kim M. Clark-Langone, Genomic Health; Carl Millward, Genomic Health; Steven Shak, Genomic Health **Honoraria:** None **Research Funding:** None **Expert Testimony:** None **Patents:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Greg Yothers, Michael J. O'Connell, Mark Lee, Margarita Lopatin, Soonmyung Paik, Steven Shak, Norman Wolmark **Administrative support:** Greg Yothers, Norman Wolmark **Collection and assembly of data:** Greg Yothers, Mark Lee, Kim M. Clark-Langone, Carl Millward, Soonmyung Paik **Data analysis and interpretation:** Greg Yothers, Michael J. O'Connell, Mark Lee, Margarita Lopatin, Kim M. Clark-Langone, Soonmyung Paik, Saima Sharif, Steven Shak, Norman Wolmark **Manuscript writing:** All authors **Final approval of manuscript:** All authors

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Appendix

Recurrence Score

The Recurrence Score on a scale from 0 to 100 is derived from the reference-normalized expression measurements in four steps. First, expression for each gene is normalized relative to the expression of the five reference genes (*ATP5E*, *GPX1*, *PGK1*, *UBB*, and *VDAC2*). Reference-normalized expression measurements range from 0 to 15, with a 1-unit increase reflecting approximately a doubling of RNA. Expression of *FAP*, *Ki-67*, and *MYBL2* is thresholded at 6.5; if the result is less than 6.5, it is considered 6.5. Expression of *GADDD45B* is thresholded at 5; if the result is less than 5, it is considered 5. Genes are grouped on the basis of function, correlated expression, or both. Second, the Stromal Group Score and Cell Cycle Group Score are calculated from individual gene expression measurements, as follows: Stromal Group Score = $(BGN + FAP + INHBA)/3$, and Cell Cycle Group Score = $(MYBL2 + Ki-67 + cMYC)/3$. Third, the unscaled Recurrence Score (RS_u) is calculated with the use of coefficients that are predefined on the basis of regression analysis of gene expression and recurrence in the four development studies¹⁷: $RS_u = 0.15 \times \text{Stromal Group Score} - 0.3 \times \text{Cell Cycle Group Score} + 0.15 \times GADDD45B$. A plus sign indicates that increased expression is associated with an increased risk of recurrence, and a minus sign indicates that increased expression is associated with a decreased risk of recurrence. Fourth, the Recurrence Score (RS) is rescaled from the RS_u , as follows: $RS = 0$ if $44 \times (RS_u - 0.82) < 0$; $RS = 44 \times (RS_u - 0.82)$; and $RS = 100$ if $44 \times (RS_u - 0.82) > 100$.

Table A1. Demographic and Clinical Characteristics for Patients in This Study Compared With All Other Patients in the NSABP C-07 Trial

Characteristic	Patients in the Present Study Cohort		Patients Not in the Present Study Cohort		P
	No.	%	No.	%	
Age, years					.394*
< 60	444	49.8	798	52.6	
60 to < 70	298	33.4	473	31.2	
≥ 70	150	16.8	246	16.2	
Sex					.423†
Male	513	57.5	850	56.0	
Female	379	42.5	667	44.0	
No. of nodes examined					.143†
< 12	344	38.6	631	41.6	
≥ 12 nodes	548	61.4	886	58.4	
Stage					.629*
II	264	29.6	431	28.4	
IIIA/B	409	45.9	688	45.4	
IIIC	219	24.6	398	26.2	
T stage					.232*
T1-2	96	10.8	194	12.8	
T3	739	82.8	1,210	80.1	
T4	57	6.4	107	7.1	
MMR status					.895†
MMR-D	84	11.8	95	11.6	
MMR-P	626	88.2	723	88.4	

Abbreviations: MMR, mismatch repair; MMR-D, mismatch repair deficiency; MMR-P, mismatch repair proficiency; NSABP, National Surgical Adjuvant Breast and Bowel Project.

*P value based on Cochran-Mantel-Haenszel row mean score.

†P value based on χ^2 test.

Recurrence Score in Stage II and III Colon Cancer From NSABP C-07

Table A2. Five-Year Kaplan-Meier Estimates of Recurrence Risk by Stage, RS Group, and Treatment in NSABP C-07

Stage and RS Group	No. of Events	No. of Patients	FU		FU + Oxaliplatin	
			5-Year Recurrence Risk Estimate (%)	95% CI (%)	5-Year Recurrence Risk Estimate (%)	95% CI (%)
II						
Low	10	103	7	3 to 17	12	5 to 27
Intermediate	9	94	8	3 to 22	10	4 to 22
High	12	67	23	12 to 42	9	3 to 25
IIIA/B						
Low	31	169	19	12 to 28	17	10 to 27
Intermediate	38	138	30	20 to 42	19	11 to 30
High	40	102	43	31 to 57	31	20 to 46
IIIC						
Low	29	73	41	28 to 57	38	23 to 58
Intermediate	36	81	48	32 to 67	40	28 to 54
High	40	65	67	52 to 82	59	42 to 76

Abbreviations: FU, fluorouracil; NSABP, National Surgical Adjuvant Breast and Bowel Project; RS, Recurrence Score.

Table A3. Relationship of Each Individual Clinical and Pathologic Covariate With Risk of Recurrence Adjusting for Stage and Treatment in NSABP C-07

Variable	HR	95% CI	P
MMR: MMR-D v MMR-P	0.28	0.12 to 0.63	< .001
T stage: T4 stage II and T3-4 stage III v T3 stage II and T1-2 stage III	3.09	1.90 to 5.01	< .001
Nodes examined: < 12 v ≥ 12	1.54	1.19 to 1.98	.001
Nodes examined: continuous (per node)	0.98	0.97 to 1.00	.047
Tumor grade: high v low	1.43	1.08 to 1.87	.013
Age			
≥ 70 v < 70 years	0.78	0.54 to 1.12	.168
Continuous (per year)	0.99	0.98 to 1.01	.387
Sex: male v female	0.86	0.67 to 1.11	.249

NOTE. Eight hundred ninety-two patients were included in the analysis of MMR, T stage, tumor grade, age, and sex. Analysis of MMR included an indicator for patients with MMR not reported versus MMR-P. One patient had missing data regarding number of nodes examined, so 891 patients were included in the analysis of nodes examined as a continuous variable. For analysis of nodes as a categorical variable, the patient with missing observation was assigned to the ≥ 12 nodes group.

Abbreviations: HR, hazard ratio; MMR, mismatch repair; MMR-D, mismatch repair deficiency; MMR-P, mismatch repair proficiency; NSABP, National Surgical Adjuvant Breast and Bowel Project.

Fig A1. Recurrence-free interval by stage and treatment in National Surgical Adjuvant Breast and Bowel Project C-07. Thick lines represent fluorouracil (FU) –treated patients, and thin lines represent FU + oxaliplatin (FU + Ox) –treated patients. Blue and gold colors represent stage II and III, respectively.

Fig A2. Cumulative incidence plots for recurrence-free interval for (A) stage II, (B) stage IIIA/B, and (C) stage IIIC patients by Recurrence Score (RS) groups and treatment in National Surgical Adjuvant Breast and Bowel Project C-07. Thick lines represent fluorouracil (FU) –treated patients, and thin lines represent FU + oxaliplatin (FU + Ox) –treated patients. Blue, gold, and gray colors represent low, intermediate (int), and high RS groups, respectively.